National Cancer Institute

Germline Polymorphic Variation, Radiation, and Cancer Susceptibility

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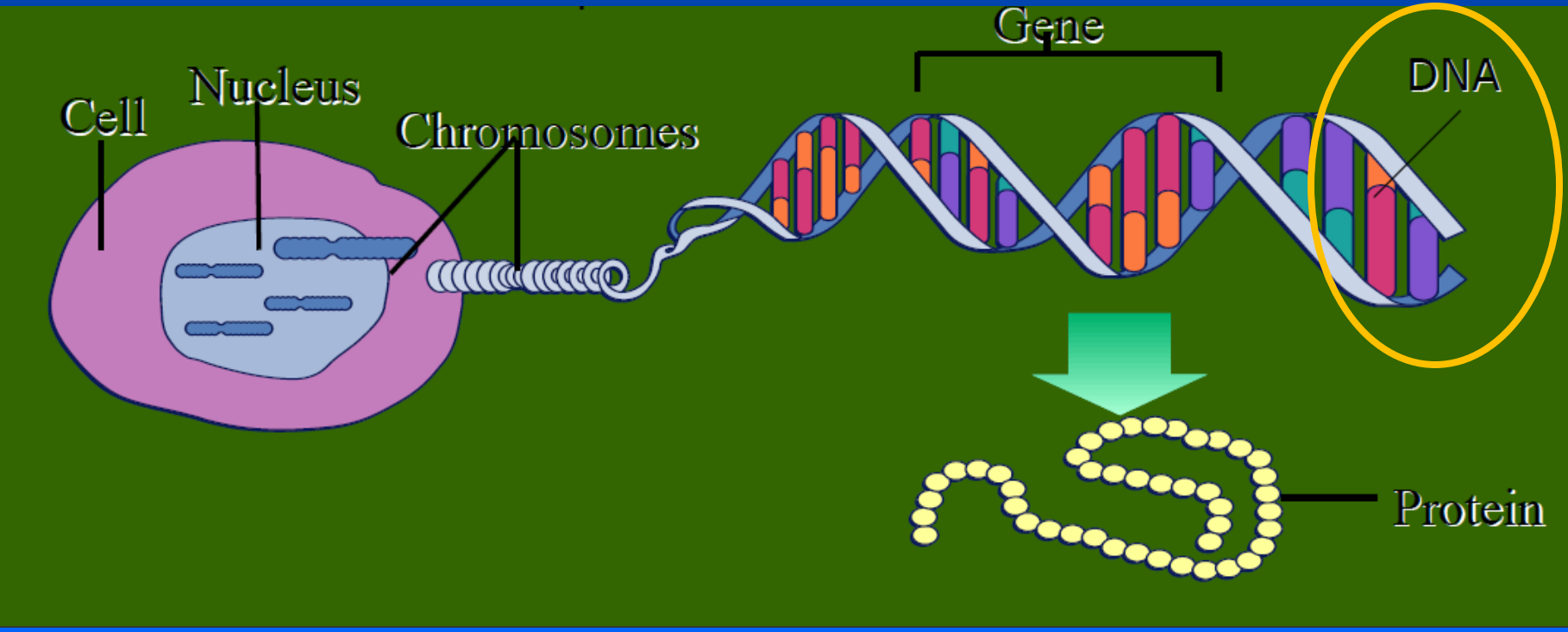
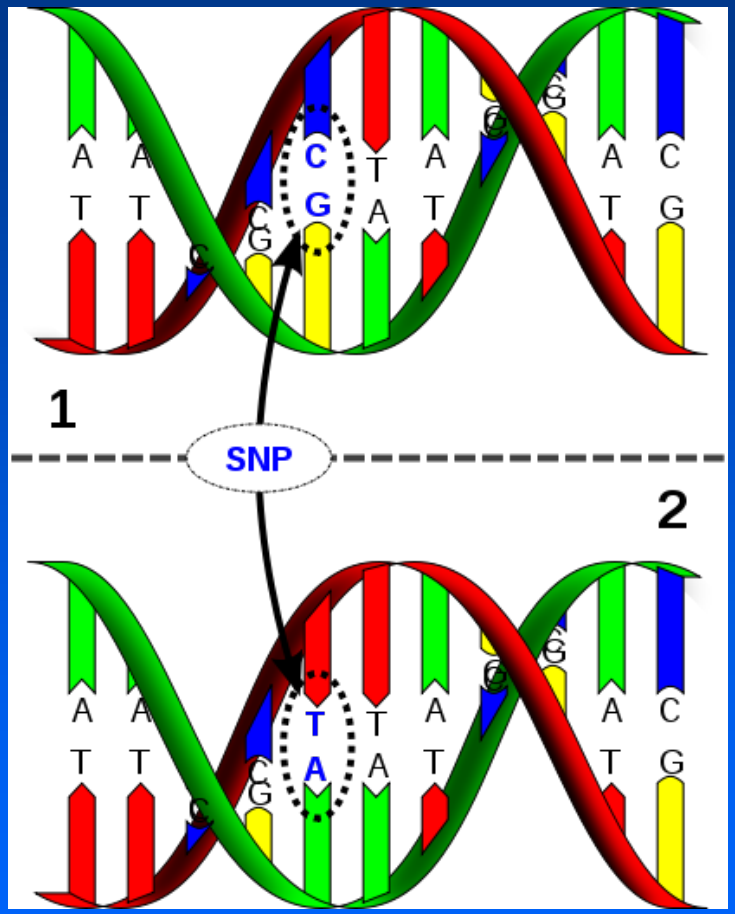
National Institutes of Health

Types of Genetic Variation

- Variation at every genetic level
  - chromosomes, genes, DNA, protein



Single nucleotide polymorphisms



- Most common genetic variation
- Each individual has two alleles
  - CC (common referent)
  - CT (heterozygote)
  - TT (homozygous variant)
- Much of the variation appears meaningless
- Some variation increases risk of cancer

Genetic Susceptibility to Radiation

- Rare syndromes with extreme radiosensitivity
- Ataxia-telangiectasia
  - Rare childhood neurodegenerative disease
  - Caused by mutations in *ATM* gene

Image courtesy Pollard and Gatti, 2009

- Cultured fibroblasts from patients three times as sensitive to radiation (Taylor et al., 1975)

How Does this Affect the General Population?

Figure 1a Increasing Incidence with Radiation Dose

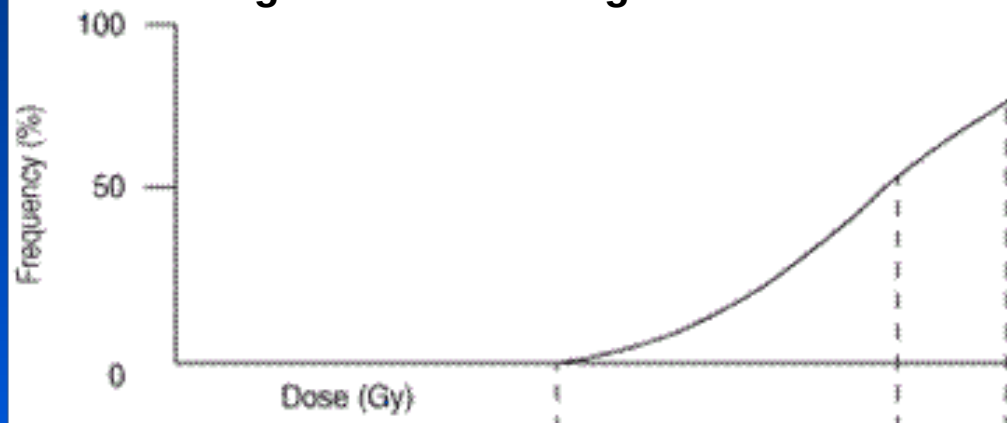
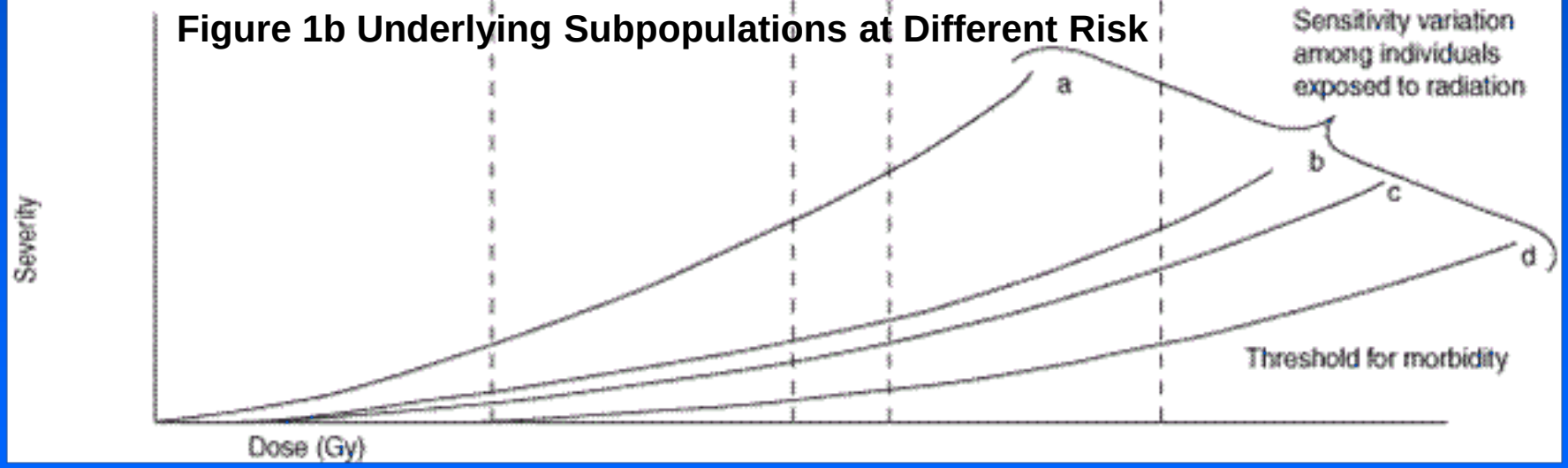


Figure 1b Underlying Subpopulations at Different Risk



Adapted from Hendry et al, 2006

The Search for Genetic Susceptibility

- Candidate gene approach
- Pathways of Interest
  - Related to Ionizing Radiation
    - Cell-cycle control/apoptosis
    - Oxidative Response
    - DNA repair
    - Immune-related
- Agnostic
  - Genome Wide Association Study (GWAS)

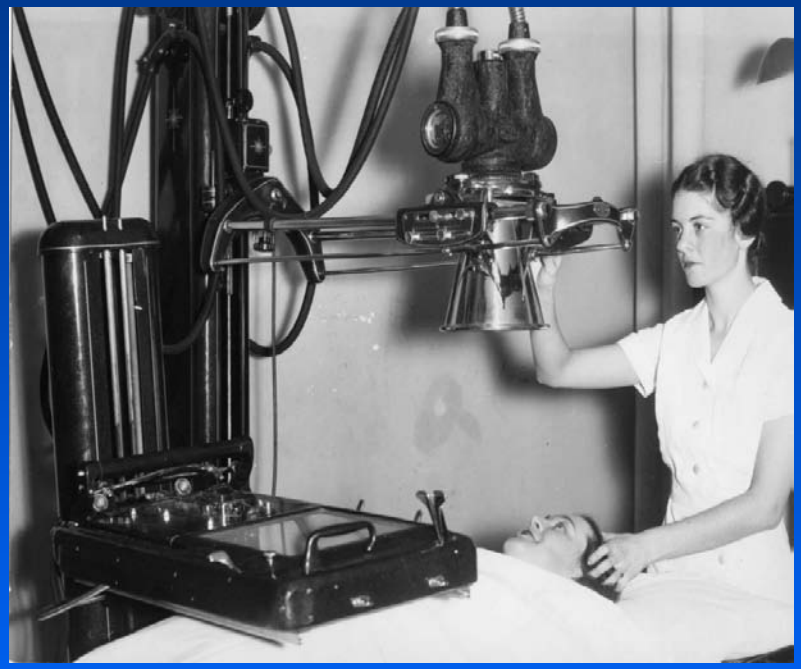
Candidate Gene Approach

- Focus on genes thought to be involved in radiation toxicity
- A few signals, but not consistent
- Limited knowledge of underlying biology; ability to query genome

DNA Repair Damage	Radiation Fibrogenesis	Oxidative Stress	Endothelial Cell Damage
ATM, NBN, BRCA1,2 H2AFX, RB1, XRCC1,4,5,6, PRKDC, LIG4, Cyclins, CDKs, CDK inhibitors	*TP53, BCL2, CASP3, TNF, ILIA, IL6, TGFB1,2,3 SMADs*	*SOD1,2,3*	*FGF2 VEGF*

United States Radiologic Technologists (USRT) Cohort Study

- 146,000 technologists certified at least two years between 1926 and 1982
- Three postal surveys
  - mid-1980's;1990's; 2000's
  - 110,000 answered at least one
- Annual follow-up of cohort
- Unique features
  - Large size
  - Predominantly female
  - Extensive covariate data



Female: 73%
Average age: 58 years
Race: 95% White
Distribution: Entire US

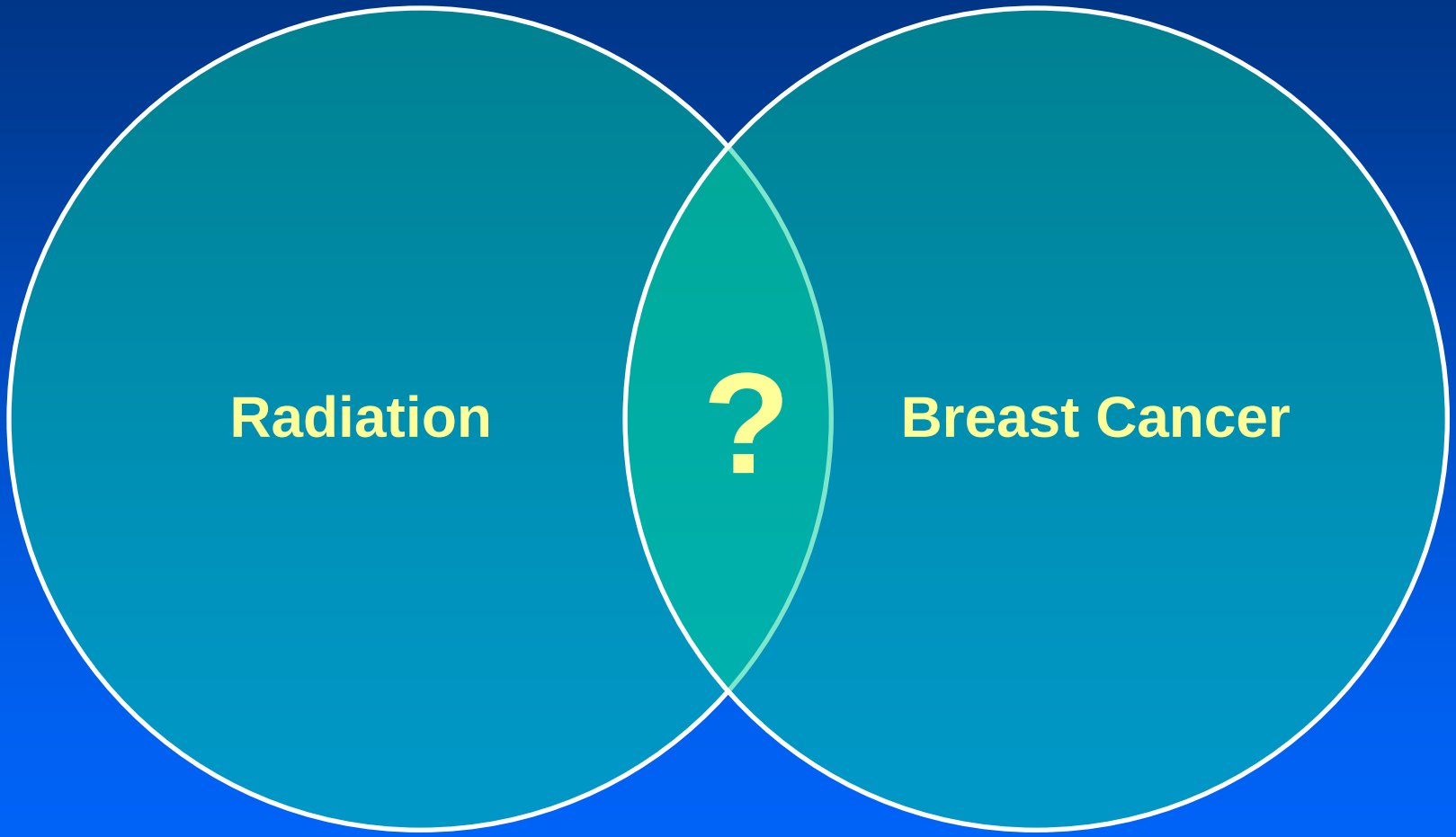
Radiation Dosimetry

- Occupational Dose (Gy)
  - simulation techniques from probability distributions describing plausible exposure range
  - probability distributions based on badge dose and literature
- Personal Medical Dose (Score, ~Gy)
  - questionnaire-based numbers and calendar time periods of procedures
  - weighted by estimates of breast doses based on literature and expert judgment
- Low mean dose
  - Occupational dose 0.03 Gy (range 0-0.59)
  - Personal medical dose 0.03 ~Gy (range 0-0.67)

US Radiologic Technologist (USRT) Nested Case-control Study of Breast Cancer

- 858 confirmed prevalent breast cancer cases
  - Year of diagnosis 1955 -1998
  - Invasive cancer (84%), DCIS (16%)
  - 62% response
- 1,083 cancer-free controls
  - Frequency matched to cases by birth year
  - 48% response
- Demographics
  - 98% Caucasian
  - Mean age at blood draw = 61
  - Provided informed consent, completed telephone interview

Risk Factors for Breast Cancer



Two main approaches

Candidate Genes in Pathways of Interest

- DNA repair
- Apoptosis
- Cell cycle checkpoint
- Oxidative damage
- Estrogen and steroid biosynthesis
- Inflammatory and immune response

"Top Hits"

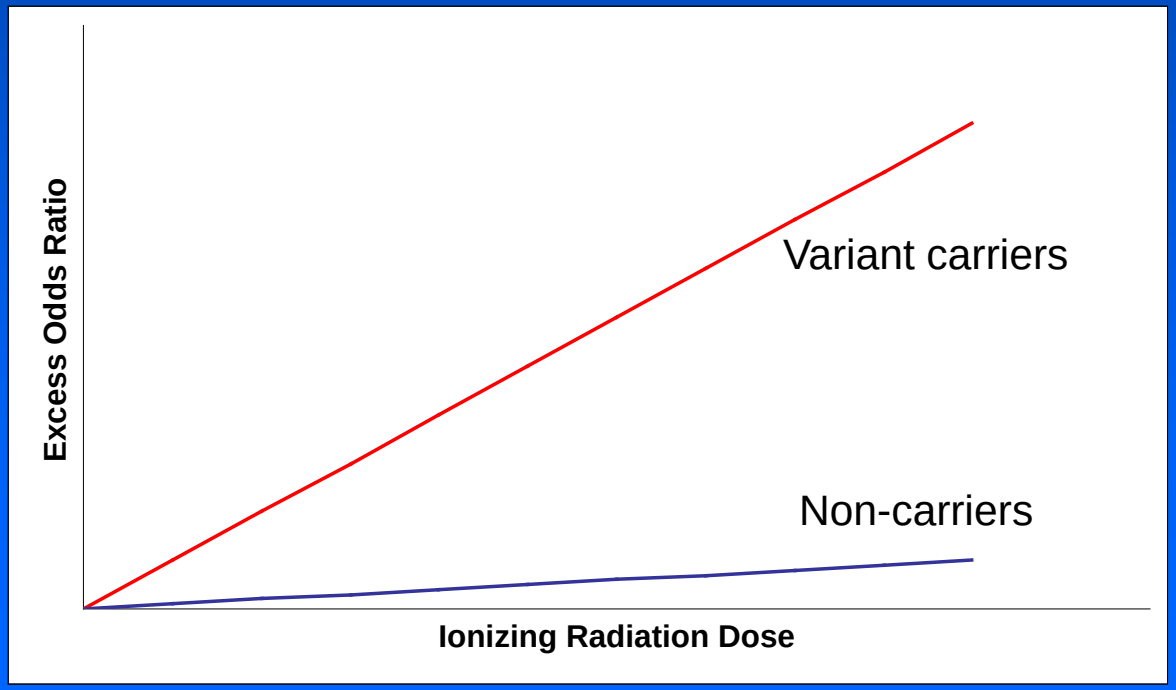
- GWAS
- Consortia

Statistical Models

- Linear term for breast dose and log-linear terms for SNPs and covariates

$$OR = \exp(\beta_1 \cdot yob1 + \beta_2 \cdot yob2\ldots)(1+ \beta_d \cdot D)$$

- Radiation-related excess odds ratio (EOR) allowed to vary by genotype



Candidate gene-radiation approach: US Radiologic Technologist Study

Pathway (SNPs, genes examined)	Gene	SNP effect	Radiation Interaction	Replication
DNA repair (61 SNPs, 21 genes)	*PRKDC*	✓	✓	?
	BRCA2	✓	N	
Apoptosis and proliferation (16 SNPs, 8 genes)	*IL1A*	✓	✓	?
	CASP8	✓	N	
Oxidative stress and inflammation (28 SNPs, 16 genes)	*PTGS2*	✓	✓	?
	IL1B	✓	N	
	IL4	✓	N	
GWAS Identified (38 SNPs)	*MRPS30*	✓	✓	?

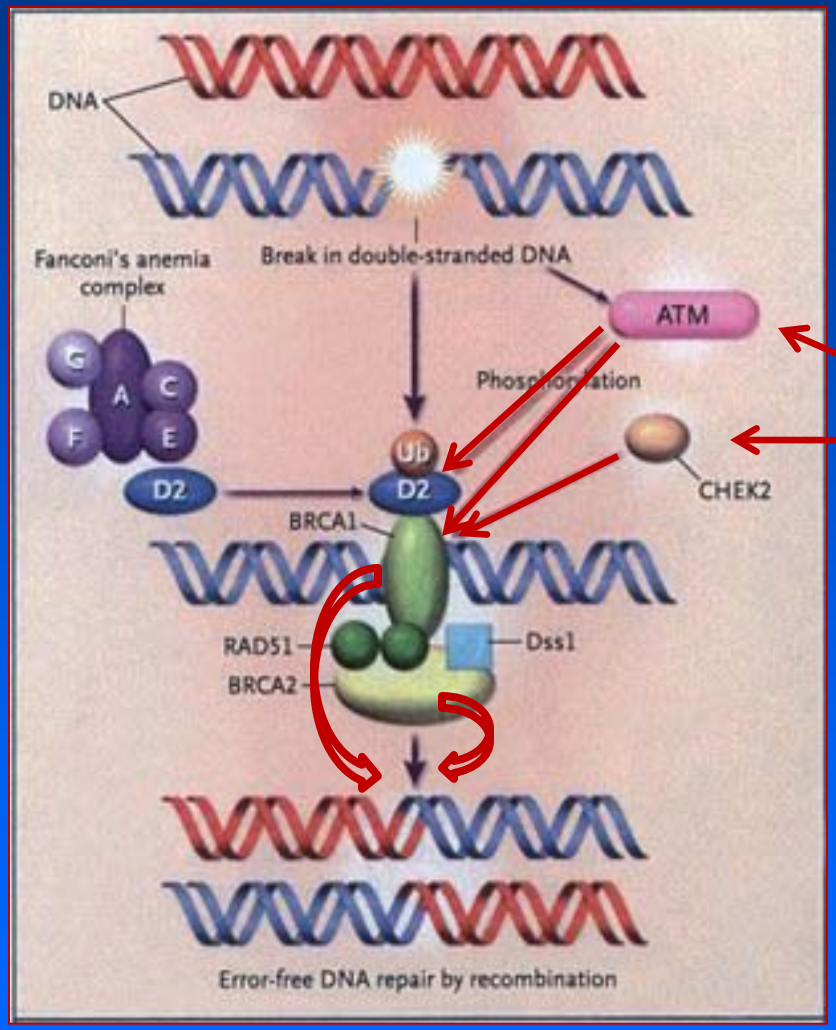
Sigurdson et al, 2007 ; Bhatti et al, 2008, 2010; Rajaraman et al, 2008; Schonfeld et al, 2010

"Top Hit" Approach
US Radiologic Technologist Study

- Select "Top Hits" from published GWAS and/or consortial studies
  - Easton et al, Nature 2006
  - Thomas et al, Nature Genetics 2009
  - Ahmed et al, Nature Genetics, 2009

- Examine with respect to radiation
  - *MRPS30*
    - 3 linked SNPs with main SNP effect
    - Evidence of interaction with occupational + personal radiation for these SNPs

Breast Cancer Susceptibility Genes



- Inactivation in genes involved in ds-DNA repair predisposes to breast (and other) cancers
  - protein kinases signal presence of ds breaks by phosphorylation
  - Induces migration to site of DNA repair

Source: Venkitaraman, NEJM 2003

BRCA1/2, Radiation and Breast Cancer

- *BRCA1* and *BRCA2*
  - carrier status confers high risk of breast cancer
  - involved in ds-DNA damage repair

Population	Exposure	Results	Citation
1,601 *BRCA1/2* carriers cohort	Chest x-rays	HR=1.75 (1.1, 2.8) *ref never x-ray	Andrieu et al, JCO, 2006
138 *BRCA1* breast cancer; 158 non-mutation breast cancer	Chest x-rays <30 yrs	OR=1.7 (0.9, 3.0) OR=1.8 (1.2, 2.9) *ref non-carrier	Gronwald et al, Br Ca Res Treat, 2008
1600 *BRCA* breast cancer cases, 1600 non-cancer controls	Age at 1st Mammography >1yr before dx	OR=1.03 (0.85, 1.25)	Narod et al, Lancet Oncol, 2006
162 *BRCA* carriers; 34 cases	No. of mammograms >1yr before enrollment	OR=0.94 (0.88, 1.00)	Goldfrank et al, 2006

Summary
BRCA1/2-Radiation and Breast Cancer

- Chest x-rays
  - Two studies show potential elevated risk
  - Self-report
  - Recall bias

- Mammograms
  - Two studies show no elevated risk overall
  - Self-report
  - Screening bias
  - Elevated risk in some categories likely to be chance

ATM, Radiation and Breast Cancer

- *ATM*
  - Involved in DNA d-s break repair

- WECARE Nested Case-Control Study
  - Cohort of unilateral breast cancer survivors
  - 708 contralateral breast cancer cases; 1,397 controls
  - *ATM* coding exons and flanking intron sequences screened for sequence variation
  - Well characterized doses from radiotherapy

ATM, Radiation and Breast Cancer

- Results
  - Variants classified: wild-type, silent, missense, splicing, truncation, common
  - No increase in risk of contralateral breast cancer with any variant type
  - Some indication of effect of radiation with missense mutations only (ERR/Gy 1.3; 95% CI 0.1-3.9)

- Rare genetic variants may influence radiation susceptibility

"Top Hit" Approach
US Radiologic Technologist Study

- Select "Top Hits" from published GWAS and/or consortial studies
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- Examine with respect to radiation
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Summary of Breast Cancer Studies

- Mainly candidate gene
- Mainly self-reported exposure
- Replication needed
- A few signals worth further investigation
  - *IL1A; MRPS30*; *BRCA1/2; ATM*

Gene-radiation: Meningioma

- Israel, 1950's
  - ~ 20,000 prospective immigrants treated with radiation for benign scalp infection (tinea capitis)
  - North Africa and Middle east, mode 6-8yrs of age
  - Mean average dose to brain 1.5 Gy

- Excess of meningioma with radiation
  - RR=9.5 (3.5-25.7) Ron et al, NEJM 1988
  - ERR/Gy=4.6 (2.4, 9.1) Sadetzki et al, Rad Res 2005

Meningioma: Family-based study (1)

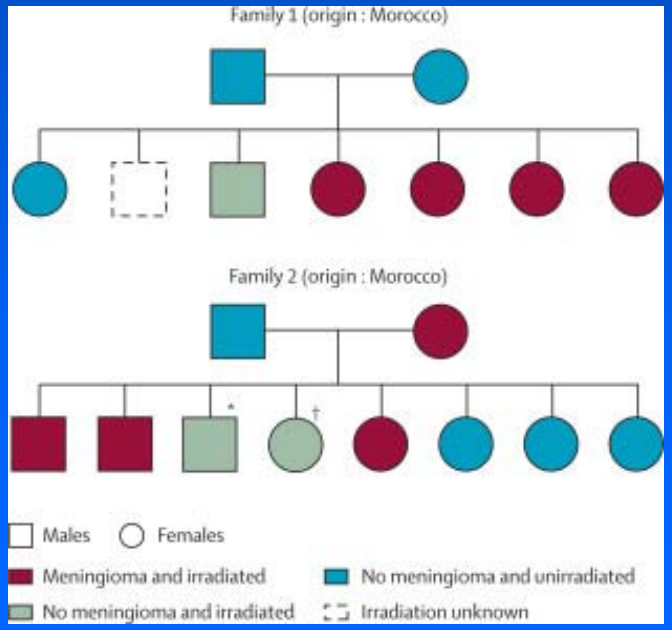
- Hypothesis: Potential aggregation of meningioma in genetically susceptible families
- 525 families from Tinea Capitis cohort
  - Classified according to index participant

	Meningioma	**No Meningioma**
Radiation	160 + 1,082 siblings	145 + 1,058 siblings
No radiation	85 + 518 siblings	135 + 863 siblings

Flint-Richter and Sadetzki
Lancet Oncol 2007

Meningioma: Family-based study (2)

- Additional 1º relatives with cancer
  - Meningioma
    - 11% in RAM families; 1% in irradiated control families (p<0.001)
  - Other irradiated sites e.g. head, neck chest
    - 10% in RAM families; 5% in irradiated control families (p=0.04)



Family 1: 5 out 7 1º relatives irradiated for TC, 4 developed meningioma

Family 2: 5 out 8 1º relatives irradiated for TC, 4 developed meningioma

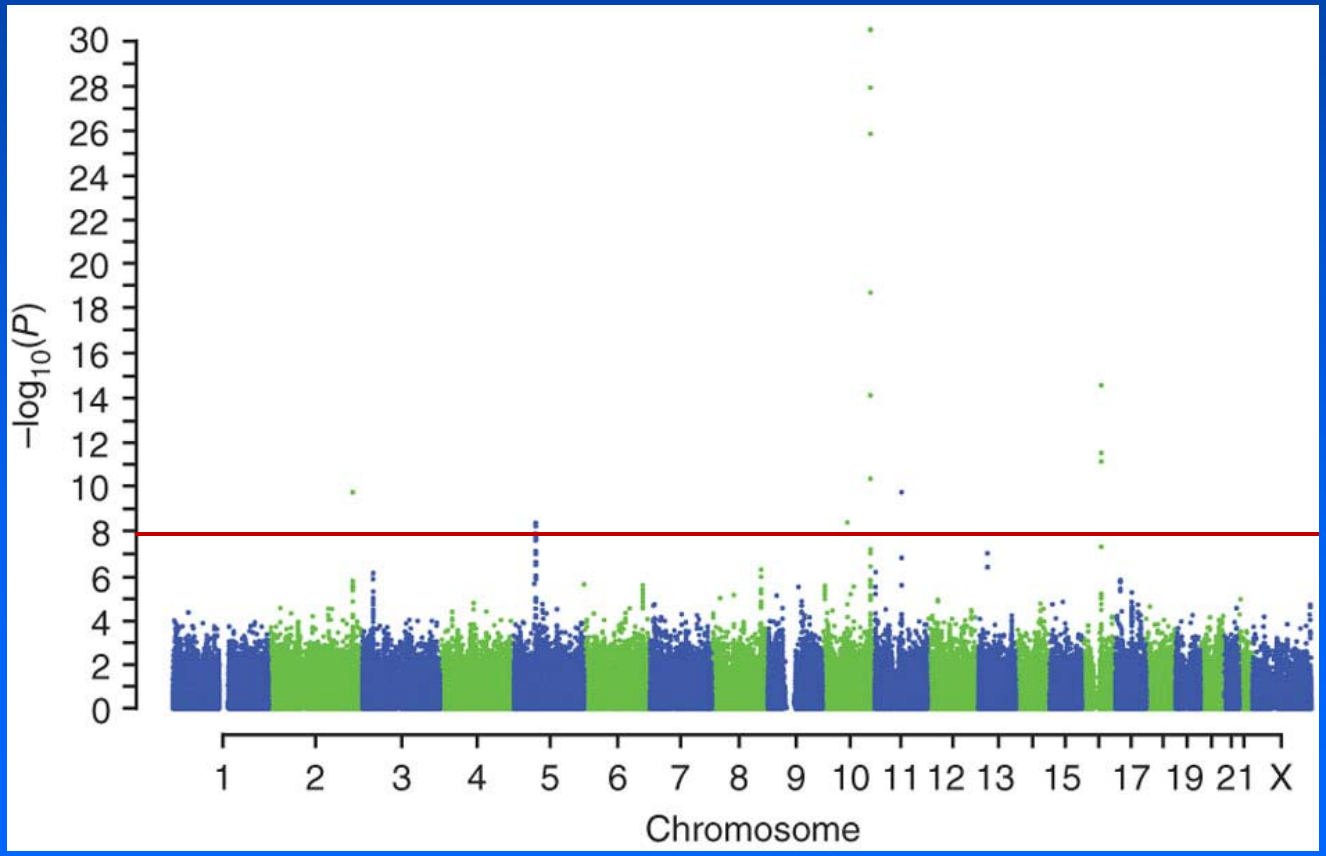
Meningioma: Family-based study (3)

- Familial clustering
  - Common exposure to environmental agents
  - Genetic factors
  - **Hereditary vulnerability to common exposure**

- Possible evidence of genetic susceptibility to radiation-associated meningioma

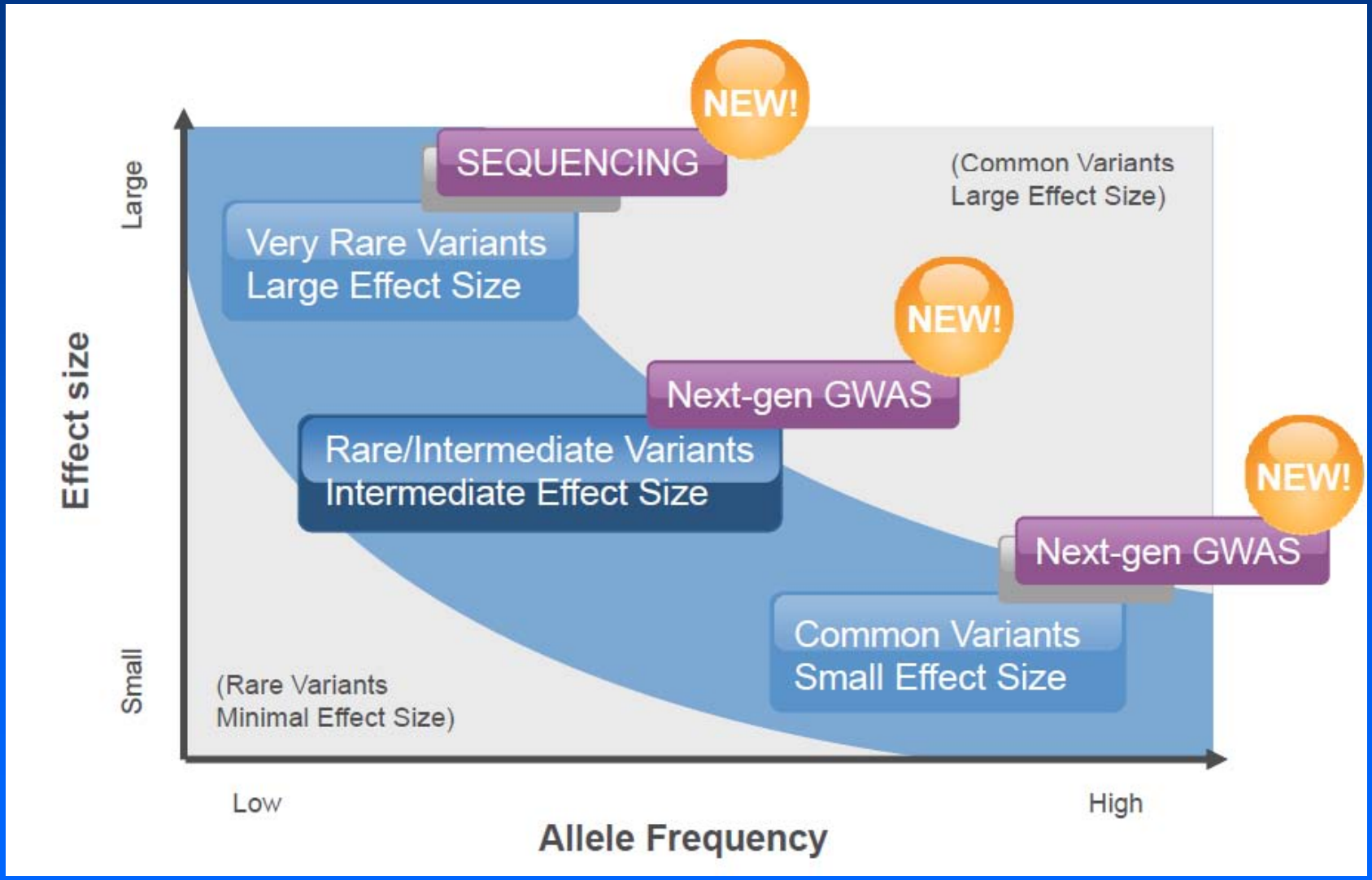
Alternate Strategy: High Throughput Genotyping

- GWAS approach has yielded important clues re: cancer etiology
- Typically 600,000 to 1 million markers across genome
- Agnostic – no assumptions about underlying biology



Breast Cancer GWAS
Turnbull et al 2010

Rapidly evolving technology



Methodological Challenges

- Setting
  - High versus low dose radiation
- Outcome
  - Cancer
  - Intermediate outcomes
- Analytical challenges
  - Power
  - Volume of data, methods need to be developed
- Replication

Radiation Genomics
A Strategy for Studying Susceptibility